The development of dorsal root ganglia and ventral horns in the opossum: A quantitative study

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SUMMARY

Numbers of cells, of mitotic figures, and of degeneration sites have been counted in dorsal root ganglia and ventral horns during the development of the opossum, from embryonic to pouch-young stages.

In the embryo, from forelimb bud stages onwards, the cervico-brachial ganglia grow rapidly, at first with very high mitotic rates (about 5%) and little cell degeneration. One day before birth, cell death is widespread through the whole series though cell numbers at all levels continue to increase. In the new-born, the growth of brachial ganglia is checked by very high local rates of cell death.

In the cord, cells of the lateral motor columns, or ventral horns arise by outward migration of cells from the basal half of the mantle layer. In the later embryo, these cells are recognizable at cervico-brachial levels during their migration, and are then accompanied by degenerating cells, the axial distribution of which foreshadows that of the lateral column of the new-born.

In the pouch-young, there is a slow loss of neurons from dorsal root ganglia and from ventral horns. This begins later at lumbo-sacral than at cervico-brachial levels.

Comparison of these results with data from other species emphasizes the variety of patterns in neural development among tetrapods.

INTRODUCTION

The problem of what factors in the nervous system control the density of neurons can be approached by counting cells at sites in embryos where discrete groups of cells are distinguishable. Such studies may provide information concerning such topics as the relationships between the development of movement and the innervation of limbs, or the role of cell degeneration in the differentiation of nerve centers.

Among lower tetrapods cell counts of the ventral horns, the motor centers for the limb muscles, were first made at selected transverse levels in Rana pipiens by Beaudoin (1955). The total number of cells in the developing lumbar horn of Xenopus laevis were counted by Hughes (1961). Similar data for R. pipiens have been given by Kollros (1968b), while Prestige (1967, 1970) has made a further close analysis of cellular events in Xenopus.

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In dorsal root ganglia, cell numbers have been counted by Coghill (1936) in *Amblystoma*; in *Xenopus* by Prestige (1965); and in the chick by Levi-Montalcini & Levi (1943); and by Hamburger & Keefe (1944). There is much scope for similar enquiries elsewhere. While the classical study on the development of the lateral motor column in the chick by Levi-Montalcini (1950) is of basic importance, the only quantitative study on that of any developing amniote is by Flanagan (1969) on the mouse fetus from day 11 to day 15; the only published data, as far as I am aware, on developing dorsal root ganglia in a mammal is by McKinniss (1936) on the human fetus. One obstacle to such studies is the greater number of cells in amniotes than in lower tetrapods. Whereas the number of lumbar ventral horn cells of adult Anura is between 1000 and 2000, the corresponding count for man is 24 600 (Sharrard, 1954).

The present pages are concerned with the growth and development of the spinal cord and ganglia in the embryo and pouch-young of the opossum, *Didelphys virginiana*. The immature condition of the marsupial at birth is of great interest from many points of view, while much scope for enquiry is offered in the correlation of nervous development with limb function, being highly premature for the forelimbs, and much delayed in the hind. Early in this study it became clear that the numbers of cells in motor centers and ganglia is considerably less in the opossum than in placental mammals. The task of counting all brachial and lumbar ganglia and ventral horns at a number of stages thus proved less formidable than had first been feared.

**MATERIALS AND METHODS**

Female opossums, both pregnant and with pouch-young, were obtained from Tarpon Zoos, Inc., Florida, early in 1970. Under general anaesthesia, the abdominal cavity of pregnant animals was opened, and embryos extracted from the uterine tubes. Pouch-young were removed from the nipples. Each specimen was staged according to the data given by McCrady (1938) for embryos, and by Langworthy (1925) and by Ulinksi (1970) for pouch-young. The three pouch-young stages selected for quantitative study were of crown-rump lengths 28, 42, and 45 mm. They were judged to be 15, 28 and 32 days p.p. respectively. The hind limbs of the first two were immobile, but those of the third showed some signs of motility. Ulinksi (1970) has discussed the difficulties in estimating the ages of pouch-young opossums.

Bouin’s fluid was used as fixative, in which embryos and new-born were immersed after opening the abdominal cavity, and also later pouch-young on transection into two halves, after first being examined for limb motility. Subsequently, fixation was seen to be good for embryos and the new-born, and adequate for cell counting in later stages, though better results would have been obtained by perfusion. Each specimen was dehydrated, infiltrated with paraffin, sectioned at 7–8 μm, and the sections stained with haematoxylin and eosin.
After preliminary study of each section series, and the identification of transverse levels through the neural axis, cell numbers were counted in dorsal root ganglia and in brachial and lumbar ventral horns under a ×40 immersion lens. Intermitotic cells in ventral horns and ganglia were counted in every fifth section, and these values were used to construct profiles of cell numbers, the area beneath which was assessed by counting squares, and the total number of cells then calculated (Fig. 4).

From birth onwards, the larger perikarya contain each a single nucleolus. As in previous studies on Amphibia (Hughes, 1961, 1962) only those cells were counted where the nucleolus was within the plane of section. This procedure avoids errors due to the spread of cells between adjacent sections more effectively than does use of correction formulae (Ebbeson & Tang, 1965; Hughes, 1969). In the nuclei of neuroblasts and the smaller neurons of pouch-young, there may be 2–3 nucleoli; only those cells were counted in which at least two nucleoli were seen within a section, a procedure which approximates to that employed with larger neurons. In the ventral horns of pouch-young from 15 days onwards, only large cells of the ventro-lateral columns were counted. In the first place, the diameters of all neuronal nuclei in this area of a selected section were measured at each stage. Where the nuclear membrane was oval in outline, the diameter of a circle of equivalent area was estimated. The numbers obtained in each size category were plotted as cumulative percentages on probability paper as described by Harding (1949). A sigmoid curve indicates a bimodal distribution, as in the present data (Fig. 1). There are thus two distinct size populations of neurons with respect to nuclear diameter, the larger of which includes the motor cells to be enumerated. As Harding showed, the point of inflection of the curve marks the transition from one population to the other. The nuclear diameter corresponding to this point was read off. In each section series, this measurement fixed the lower size limit of the neurons counted. An eyepiece micrometer in the field of view was used to check which cells were to be included. For counts which involve some element of subjective judgement, reproducibility within no closer limits than 15–20% can be claimed, a range of error only acceptable where the observed differences between stages, from which conclusions are drawn, are on a much larger scale. Ganglia were counted on one side only, and the main conclusions derived from a study of the data checked by reference to the other side. In the spinal cord, cells were counted on both sides; the results agreed closely in both. Data for one side are here presented (Fig. 5).

Degenerating and dividing cells were also enumerated and were counted in every section. Dividing cells were scored only where the greater part of the mitotic figure was included. Counting of degenerating cells is less satisfactory. Their stage of degradation varies from one with an early pycnotic nucleus and the nuclear membrane still intact, to disorganized clumps of dense phagocytosed material within a macrophage-like cell. The difficulty is not met by
Fig. 1. Nuclear diameters (ordinate) in selected sections through ventral horns at 15 (circles) and 32 days (squares) p.p. respectively, plotted as cumulative percentages (abscissa). The distribution of each is bimodal, with the transition between populations with small and large nuclei at 6.5 and 11 μm respectively.

attempts to trace one site through adjacent sections, for there is a strong tendency for cells to degenerate in groups. It can be avoided by calling them all ‘degeneration sites’, admitting that they do not necessarily correspond with single instances of cell death.

RESULTS

The dorsal root ganglia

(1) Segmentation. According to Voris (1928), the first two cervical nerves in the opossum give rise to the hypoglossal, while ganglion C2 has other muscular branches. Cutaneous nerve innervation of the neck comes from C2 and C3. The first spinal ganglion must thus belong to C2. At stage 28, the first group of ganglion cells is much smaller than the succeeding members of the series, and contains a larger number of degenerations (Fig. 3a). It thus seems to be a vestigial ganglion of C1. Identification of spinal ganglia according to these considerations at later stages was confirmed by tracing the main nerves into the limbs. The cervical, brachial, lumbar, and sacral plexuses of the opossum are generally similar in the distribution of their component nerves to those of placental mammals.

(2) Histological. McCray (1938) states that at stage 25 (13 somites, day 9½) the neural crest ‘in the somite region is aggregated segmentally’. At stage 28
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The ganglia consist of discrete groups of cells only as far back as T 2 , caudal to which they are represented by a continuous strip of neural crest cells. The ganglia are made up of cells with rounded nuclei, each containing from one to three nucleoli. The surrounding cytoplasm stains evenly with eosin, and appears at the magnification used as a continuous matrix in which nuclei are embedded. No signs of any distinction between neuronal and glial cells are yet apparent, nor are axons yet visible within these ganglia or beyond, though ventral root fibers from the neural tube are already present.

At stage 33 (day 12½), bundles of neurites can be traced from each pole inwards to the cord, and distally to join the corresponding motor root. Blood vessels have entered the ganglia. The nuclei of cells within each ganglion now vary in both size and shape. The largest are round or ovoid, and about 10 μm in diameter. Their nuclei are eccentrically placed in a distinct cytoplasm which stains clearly with eosin. Such cells are found throughout the ganglion, and are not concentrated at either pole. They are found most frequently at cervical levels, and in the limb segments make up about one fourth of the cells present. They become less abundant at thoracic levels, while no cells at lumbar levels have yet reached this stage of differentiation. Less mature cells of the ganglia at all levels have smaller ovoid nuclei and a less conspicuous envelope of cytoplasm. The nuclei of all developing neurons at this stage have generally a single nucleolus.

Other nuclei belonging to future Schwann cells, satellite cells or fibroblasts are elongated. They are found more frequently on the inner surface of the ganglion, and at the dorsal pole among the fibers of the central process. Distal to the ganglia are seen more differentiated Schwann cells with elongated nuclei up to 18 μm long.

At birth (stage 35, day 13½), the larger neurons are concentrated towards the ventral poles of the ganglia. Cells are arranged in columns such as Prestige (1965) has described in the lumbar ganglia of *Xenopus* tadpoles at stages with motile hind limbs. Some of the more mature cells at the ventral pole of cervical and thoracic ganglia are surrounded by an investment of satellite cells which at this stage are rarely seen at lumbar levels. Otherwise, little difference is seen with respect to the development of viable cells throughout the series of ganglia.

In later pouch-young opossums, the ganglia are wholly post-embryonic in appearance at all levels. The neurons differ in size, but even in the smallest, cytoplasm is evident. All neurons are enveloped by satellite cells. A considerable volume of the interior of each ganglion is occupied by nerve fibers.

(3) Quantitative. The numbers of cells counted in each ganglion are shown in Fig. 3. The totals for the brachial and lumbar series are given in Fig. 6, which demonstrates that in the embryo and the new-born, cell numbers in all ganglia are rising rapidly. These figures can be compared with the corresponding counts of dividing and degenerating cells. At stage 28, the numbers of degenerations in ganglia C 2 caudalwards are low enough to disregard. The general mitotic
Fig. 2. (A) Section through basal plate of spinal cord in brachial region at stage 33, showing small dark and large lighter nuclei in mantle layer. The latter belong to cells migrating through mantle layer. Arrow: degenerating cells. Marker indicates 100 μm. (B) Section through ganglion C₈ of new-born opossum, showing loss of cells by degeneration at ventral pole of ganglion (arrow) with pycnotic nuclei of satellite cells. Marker indicates 100 μm. (C) Section through basal plate of spinal cord of new-born showing compact ventro-lateral group of motor cells (arrow) at margin of mantle layer. Marker indicates 100 μm.
rate is about 5% (Fig. 3a). An estimate whether this figure is sufficient to account for the very rapid growth of the ganglia at that time (Fig. 6) can be based on the consideration that if the duration of mitosis is 1 h, and if, furthermore, one cell enters division every hour, then the rate of addition of new cells is 1/h. In cultures of the mouse spleen, Fell & Hughes (1949) measured mitotic times of 43–90 min; if we assume, in the absence of information specific to the opossum, that its duration of mitosis is also of the order of 1 h, then the number of divisions at stage 28 may be adequate to account for the rates of increase in cell numbers, which are of the order of 5%/h, and would thus not require any further recruitment of neural crest cells to the ganglia. So rapid a rate of growth is requisite for the complete development of a system of dorsal root ganglia in as short a period as 4 days.

At stage 33 the numbers of degeneration sites throughout the ganglia are greater than the number of mitotic figures, with a general percentage division rate of about 1-5 (Fig. 3b). Yet the lumbar ganglia are then doubling in cell numbers in 36 h. These facts indicate either that the duration of cell degeneration is much longer than that of mitosis, or that a greater number of degeneration sites are counted than the actual number of dying cells.

The general mitotic rate in a ganglion may be higher than that of its neuroblastic cells, as McKinnis (1936) pointed out for human fetal ganglia. In the ganglia of embryo and new-born opossums, all types of cells were counted, and these numbers include future Schwann and satellite cells as well as neuroblasts. The plane of section of the new-born animal studied is nearly transverse to the axis of the lumbar ganglia, and so the central process fibers on their way into the cord are grouped towards one end of the section sequence. Here, as Fig. 4 shows, mitoses are concentrated among future Schwann cells, whereas degenerations are distributed evenly throughout the whole cell mass. In the newborn, cell degeneration is on a hitherto unknown scale in ganglia C7, C8, and to a lesser extent T1. In the two former, up to 700 pycnotic nuclei are seen in each (Fig. 3c), largely concentrated towards the ventral pole in the area of the largest and most mature cells (Fig. 2B). Open spaces are left in this region, which is littered with cytoplasmic debris, and pycnotic nuclei, mostly of satellite cells. Fig. 3(c) shows that this high incidence of degeneration within brachial ganglia is associated with a very low mitotic rate. It may be that among the cells which degenerate are some which had already entered division, a possibility which is suggested elsewhere in the ganglia by the presence of groups of clumped chromosomes, where it is sometimes not certain whether they are to be scored as degenerations or metaphases, though prophase nuclei are always exquisitely clear.

Though cell numbers have increased in the brachial ganglia since stage 33, mainly in C8–C6, and in C9 and T1, the growth of the lumbar ganglia, particularly of L6, has been considerably greater. This difference between brachial and lumbar ganglia is maintained in later pouch-young.
Fig. 3. Total cell numbers (squares) in successive dorsal root ganglia as shown, and numbers of degeneration sites (triangles) to scale on left together with percentages of mitotic figures (circles) to scale on right for: (a) C₃ to T₂ at stage 28; (b) C₄ to T₄ and T₁₃ to S₁ at stage 33; (c) same ganglia as in (b) for new-born. (d) Shows total numbers of neurons in ganglia at 15 days p.p. (open squares) and at 28 days p.p. (circles).
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Fig. 3c and 3d. For legend see opposite.
Fig. 4. Ganglion L₄ at stage 33. Total numbers of cells plotted for every 5th section (squares) with numbers of degeneration sites (triangles) in successive groups of 5 sections, both to scale on left. Local percentages of mitotic figures (circles) for each successive group of five sections, to scale on right. Abscissa: section number. The horizontal dotted line (top of figure) indicates the limits of the central process fibers issuing from the ganglion.

From 15 days p.p., counts are of neurons only (Fig. 3d), and so the figures for early and later stages are not directly comparable (Fig. 6). It can be seen, however, that at lumbar levels the number of neurons at this stage is greater than that of total cells in the new-born; the lumbar ganglia are thus still actively growing, despite some fall in their mitotic rates since stage 33 (Figs. 3b, c).

Sometime about 15 days p.p., a second and much slower phase of cell loss
begins in the brachial ganglia (Fig. 6). Between this stage and 28 days p.p. cells disappear at a rate of about 1 cell/1000/h. However, no degenerating neurons were recognized in sections under the microscope, and such a rate of cell death must result only in an inconspicuous proportion of pycnotic nuclei.

The spinal cord

*Embryos.* As with the dorsal root ganglia, stage 25 (day 9½) marks also the origin of the neural tube, when the neural folds ‘come together and fuse – just caudal to the otocysts’ (McCrady, 1938). At stage 28 (day 10½), the anterior neuropore is closed, while posteriorly the neural tube opens into a flat rhomboidal sinus. Abundant mitoses are seen in the ependymal zone of the neuroepithelium. Towards its outer surface, there are lacunae between adjacent cells, probably exaggerated by shrinkage during preparation. Within these spaces, sparse longitudinal fibers can be seen, the first signs of the white matter of the cord. In cellular arrangement, the cord then resembles a 50 h chick or 9-day mouse fetus, but a marked distinction from these is the prominence of ventral root fibers, both within the cord, and laterally for some distance into the mesenchyme. They are particularly clear at the level of C₆.

Within 2 days there is considerable differentiation within the cord. At stage 33 (day 12½) three zones are evident, the ependymal and mantle layers, and the outer white matter. The ependymal layer is wider in the alar than in the basal plate, and mitoses are more frequent within the former. The nuclei of ependymal cells are ovoid, and relatively dense; they contain a single concentrated nucleolus. In the mantle zone the nuclei are larger and more rounded, and have less conspicuous nucleoli. Within this zone, an alar and a basal lamina can be distinguished. The alar nuclei are uniform in appearance, whereas there are two types within the basal lamina, one large and lightly stained, the other smaller and much denser. Both types are evenly distributed throughout the region (Fig. 2). No degenerating cells are seen in the alar lamina, while basally they are abundant.

Fig. 5(a) shows the incidence of degeneration sites counted in every section through the basal lamina from rostral levels caudalwards, expressed as numbers in successive groups of ten sections. These rise to a maximum between the levels of C₈ and T₃, and then subside to a relatively low level from T₄ onwards. Caudal to S₁ no degenerations were counted. Within the cord, pycnotic debris is clearly seen to be within phagocytic cells.

In the white matter at stage 33, there are also recognizable features. The incoming fibers from the dorsal root ganglia give rise to a dorso-lateral funiculus bounded on each side by cells which extend to the surface of the cord. The dorso-lateral funiculus is thus distinct from the remainder of the white matter, which forms a continuous investment round the cellular zone of the cord laterally and ventrally. Within this layer of fibers, a ventral commissure is conspicuous,
Fig. 5. (a) Numbers of degeneration sites in successive groups of ten sections in basal lamina of mantle layer at a stage 33. (b–d) Cells in ventro-lateral motor columns, plotted for every 5th section, (b) cervico-brachial column in new-born; (c) and (d) cervico-brachial and lumbo-sacral columns at 15 and 28 days p.p. respectively. Abscissa: segmental levels as shown.
and opposite the basal zone of cells, ventral root axons make their way through masses of longitudinal fibers.

The cord at cervical and brachial levels in the new-born (stage 35) is now much broader than at earlier stages, due to continued cell production by the ependyma, which is markedly depleted of cells basally, where the outward movement has been on a greater scale. At brachial levels, the outer margin of the mantle layer shows evidence of still further lateral migration, where separate columns are seen of closely massed cells, one lateral, the other ventral. The former is the larger and consists of mainly bipolar cells, with the largest nuclei in the cord (Fig. 2C). Their maximum diameter is about 12 μm. This column is restricted to brachial levels, and extends from C₃ to T₃, with a plateau in numbers at C₈ to T₁ (Fig. 5b). Comparison of Figs. 5(a) and (b) shows that the axial profile of this lateral column resembles in shape that of degenerating cells in the basal lamina of the mantle layer at stage 33. Integration in appropriate units of the areas at brachial levels of the profiles of counts in Figs. 5(a) and (b) shows that whereas in the new-born 8940 cells have migrated laterally to form the ventro-lateral column, early in their journey through the mantle layer at stage 33 there were 340 degeneration sites among them, though others may also undergo cell death between these stages.

The ventral column is made up of cells with nuclei less distinct in size from those of the mantle layer, but with a greater density of cytoplasmic material. It extends further caudally than the lateral column. Neither column is yet distinct at lumbar levels, though the lateral column can be traced in a few segments.

Degenerations within the cord are now infrequent, and are rare both in the mantle layer and in the brachial cell columns. Where at lumbar levels lateral motor neurons are recognizable, a small number of degeneration sites are seen medially in the mantle layer. Thus while the brachial column is foreshadowed in the distribution of pycnotic nuclei among migrating cells at stage 33, the lumbar column in our material is not preceded by a recognizable pattern of degenerations, though it is possible that this might be seen at the intermediate stage 34.

In the white matter, the dorsal funiculus has now extended dorsally and inwards almost to the mid-line, but is largely separated from other longitudinal tracts by the cellular dorsal horn, which extends nearly to the surface of the cord.

Later pouch-young. The cord is wholly post-embryonic in appearance, with a narrow central canal, deep median septa, and the extension of the white matter round the whole circumference. The dorsal funiculus has been heavily invaded by cells with small, dense nuclei, presumably oligodendrocytic. The motor columns consist of large neurons dispersed among smaller cells. Of the latter, some have clear nuclei, which can be identified as small neurons, while others belonging to glial cells have still smaller dark nuclei. As before, two main columns of large neurons can be distinguished, one lateral or ventro-
lateral, and the other medial, made up of rather smaller perikarya. The latter can be traced throughout the length of the cord, though it is less conspicuous at lumbar levels. It resembles the motor columns of other tetrapods in the same position which are concerned with the innervation of axial muscles.

The more lateral cells at cervico-brachial and lumbo-sacral levels constitute the ventral horns. At 15 days p.p., the brachial horn extends from the levels of ganglia C₃ to T₂ (Fig. 5c), but by 28 days has retreated rostrally to the level of C₆. At 15 and 28 days p.p., the lumbar horn extends from L₃ to S₁, but retracts to that of L₆ by 32 days p.p. The nerve roots, particularly the lumbar series, join the cord at levels several segments more cranially.

As described earlier under Material and Methods, neurons within the older ventral horns form bimodal populations of cells with respect to nuclear size, only the larger of which were enumerated. Counts were made within these ranges of nuclear diameter; at 15 days, 6.5–10 μm; at 28 days, 8–16 μm; and at 32 days, 11–20 μm. In the latter only the lumbar horn was counted. Most of these large cells are motor neurons, though some may be interneurons. Both are derived from the closely packed cells of the lateral columns of the new-born. The numbers of cells counted at each stage (Fig. 6) are correlated with the axial
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lengths of the ventral horns. Loss of cells in the brachial horn begins soon after birth, but not until 28 days p.p. in the lumbar horn. As in the dorsal root ganglia of pouch-young, the decline in cell numbers within the ventral horns is too slow to be accompanied by microscopically recognizable signs of cell death.

The ventral horns of pouch-young here studied show little or no signs of the subdivision into cell columns which Voris (1928) described in the adult opossum, though in the lumbar horns cells take up a more lateral position caudalwards at the level of the main limb nerves.

DISCUSSION

The causative factors which control differentiation within the spinal cord and ganglia are not all of one kind. Some are intrinsic to the developing central nervous system, and vary in operation from one level to another. Thus in the *Xenopus* tadpole at an early limb-bud stage, the future limb ganglia then contain a relatively large number of uniformly small cells, while the ganglia at non-limb levels are made up of smaller numbers of cells, some of which are of relatively large size (Hughes & Tschumi, 1958).

At later stages, those centers opposite the limbs are subject to influences which emanate from the periphery, which establish the final size differences between ganglia at limb and non-limb levels. Their operation has been demonstrated by numerous studies on the hypoplasia of developing spinal ganglia and ventral horns caused by limb ablation (Shorey, 1909; Hamburger, 1934) and of hyperplasia in ganglia resulting from grafting of supernumerary limbs (Detwiler, 1936; Hamburger & Keefe, 1944). For these investigations, urodeles (Detwiler, 1936), Anura (Prestige, 1965, 1967, 1970) and the chick embryo (Hamburger, 1934, 1939, 1958; Hamburger & Keefe, 1944) have been used.

In the present observations on the opossum, identification of such influences is limited by the absence of experimental evidence. However, the operation of intrinsic factors can be surmised at stage 28 from the generally high mitotic rates of cervical compared with thoracic ganglia. A profile of their mitotic rates when serially plotted (Fig. 3a) resembles that of the corresponding cell numbers in the pouch young (Figs. 3b–d). Regional differences are thus recognizable from the first and do not arise through differential cell degeneration, as in the cervical ganglia of the chick (Hamburger & Levi-Montalcini, 1949).

The forces which mold the developing nervous system do not act uniformly from one group of animals to another. A major difference can be discerned in larvae and in other developing tetrapods between their operation in the Anuran. In the tadpole, the limbs develop relatively late under the influence of mounting thyroxine levels towards metamorphosis (Etkin, 1963; Kollros, 1968a). The ventral horn, with its high initial number of small cells, becomes sensitized by thyroxine to control by the limb, which regulates the subsequent rate of cell death in the ventral horn, and its rate of decline in total cell number. In *Xenopus* tadpoles, degenerating cells are first seen in both lumbar ventral horns.
and spinal ganglia at stage 53 of Nieuwkoop & Faber (1956) shortly after digits become visible, and the limb shows signs of motility. Among the lumbar ganglia, the effect on total cell numbers is to inflect a hitherto exponentially rising curve ultimately to the extent of a downward turn at metamorphosis (Prestige, 1967).

How far hormones enter into the control of the early phases of neural development in amniotes is unknown, with the striking exception of the evidence provided by the opossum. At birth, the anterior lobe of the pituitary and the thyroid are only at an epithelial stage, and the adrenal cortex is rudimentary (McCray, 1938). Moreover, the transitory and wholly epithelial placental relationship between yolk sac and endometrium argues against any effective transfer of maternal hormones. It is thus likely that neural development in the opossum embryo is uninfluenced by endocrines. Mitosis and cell degeneration among neuroblasts in cord and ganglia must be intrinsically regulated, with moreover a rate of growth in cell numbers far higher than in \textit{Xenopus} larvae.

In the nervous system of the embryo opossum, cell death shows certain peculiarities:

(a) Degenerations among future brachial horn cells during their migration through the mantle layer of the cord. This feature is in contrast with the mouse, the only other mammal for which information is available, where, as Flanagan (1969) has shown, migration is completed a day before degeneration begins among the ventral horn cells, then already in their final ventro-lateral position.

(b) The abrupt and localized degeneration of larger cells in the brachial ganglia seen after birth. If we assume that the cells lost belonged to sensory pathways wholly concerned with forelimb action at birth, and which thus become redundant, the question then arises whether these neurons were exteroceptive or proprioceptive in function. The arguments which suggest the former are first that these cells correspond in position to the ventro-lateral group in embryonic chick ganglia, which there is reason to regard as exteroceptive (Visintini & Levi-Montalcini, 1939) and secondly, that the muscular system of the embryo and early pouch-young is little differentiated, with all muscle fibers at the myotube stage, one which generally precedes the appearance of muscle spindles by a considerable margin. It is not the least of the remarkable features of opossum parturition that the unaided climb to the pouch should be wholly due to musculature at so primitive a level of organization.

(c) The slow loss of neurons in ventral horns and ganglia in the later pouch young. This phase of neural development corresponds in this respect with the decrease in cell numbers of ventral horn and ganglionic neurons in the Anuran tadpole before and immediately following metamorphosis, and is possibly in both under endocrine influence. In \textit{Xenopus}, there is evidence that this phase of ventral horn differentiation is related to the development of function in the hind limb (Hughes & Prestige, 1967) whereas the special feature of the opossum pouch-young in this respect is that the two pairs of limbs are then at such dis-
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parate stages of motility. The forelimbs have already carried out a major assignment, while, as Langworthy (1925) described, it is not until after 50 days in the pouch that the animal is able to use the hind limb effectively in standing or walking. Yet as Blincoe (1962) has shown, the nerves of the lumbo-sacral plexus can be traced to their muscles at 4 days after birth.

Thus while the slow loss of neurons in the ganglia and cord of the pouch young is in advance at brachial over lumbo-sacral levels, this difference is not related to functional considerations, for it follows motility in forelimbs, and precedes the effective action of the hinder pair. The neurological basis for the slow development of hind-limb function remains for the electron microscope to explore, in the way in which Bodian (1966a, b) has demonstrated how the unfolding of motility patterns in the limbs of the *Macaque* fetus is closely correlated with the development of synapses round the motor neurons of the cord.

These considerations illustrate how comparative enquiry has still much to offer in research into neural development, particularly in regard to the incidence of cell death, the significance of which in different contexts is still largely obscure. Here such studies can illustrate the diversity of causal relationships, and discourage premature generalization.

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